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November 17, 2004

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APPLICATION NUMBER: 60/510,358 FILING DATE: October 10, 2003

RELATED PCT APPLICATION NUMBER: PCT/US04/33194

Certified by



Jon W Dudas

Acting Under Secretary of Commerce for Intellectual Property and Acting Director of the U.S. Patent and Trademark Office

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PTO/SB/16 (08-03) Approved for use through 07/31/2006. OMB 0651-0032

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Ge Ming		55 South Kukui Street Apt 2810 Honolulu, HI 96813					
Additional inventors are being named of	bered sheets attached						
	TITLE OF THE INVENTION						
Composition and Methods for Ce							
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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

USE UNLY FOR FILING A PROVISIONAL APPLICATION FOR PATEN1
This collection of Information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

PTO/SB/17 (10-03)

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Inventor: Ge Ming Lui Patent Owner: Cellular Bioengineering, Inc. 1946 Young Street Suite 480, Honolulu, HI 96826 Phone: 808-949-2208

Patent 5: Composition and Methods for Cell Culturing

BACKGROUND ON VARIOUS STEPS

Title: 2E - A Novel Surface for Cell Growth, Coating, Expansion, and Transplantation

Background

Brief description of what this patent describes:

This patent describes the method of coating a polymer surface with Diamond Like Carbon (DLC) to render it useful as carrier for cells derived from neural origin. What problem(s) does this address?

It is very difficult to induce neurons to attach and grow onto polymer in tissue culture without the coating of neurotropic substance on the surface of the polymer.

How is this different from what's been done by others?

Attempts are made to grow neurons on bare polymer surface without much success. What future applications might this have?

The polymer, either in the form of thin sheets or small beads, can be coated with DLC and be used as cell carriers for transplantation of neurons.

Specific Methods and/or Compositions

the May Griffith and the Lawrence Berkeley patent.

Step-by-step preferred method and/or description of composition:

DLC coating onto polymer will render it useful for carrying cells derived from neural origin. In combination with embedding a cocktail of attachment proteins-growth factors inside the matrix of the polymer, it may sustain the growth of nerve cells for a short period of time, up to several days. The polymer, either as a bead or a sheet, can act as a carrier for the implantation of nerve origin cells. The implantation of DLC surface was done using a filtered vacuum-arc system. The vacuum-arc is formed by a high current discharge between two electrodes in vacuum. This produced abundance of carbon plasma from the cathode material., which carried the arc current. A repetitively plused vacuum-arc-plasma source was used with a pluse length of 5 ms and a repetition rate of 1/second. Any macroparticles was removed by a 90 degree magnetic filter mounted on a curved "magnetic duct" which stops line of sight transmission of macroparticles while allowing the transmission of plasma by virtue of an axial magnetic field. The substrate was mounted on a grounded holder about 10 cm from the duct outlet. The plasma gun or duct were at or near ground potential (within about 20 V). The film deposition is an energy deposition, resulting in a high quality, hydrogen free, diamond like carbon, not amorphous carbon or graphite.

The IP position in this case is the carrier function of the combination product, which is different from both

Alternative steps or materials to address potential problems or if certain materials are not available:

Biodegradable polymers, when available, can be a more appropriate carrier for cell transplantation in the eye and the brain.

Better methods or compositions if new materials or complementary methods become available:

To embed the polymer with adhesive molecules/growth factors may enhance the biopolymer's ability to support neuron attachment and growth.

Inventor: Ge Ming Lui Patent Owner: Cellular Bioengineering, Inc. 1946 Young Street Suite 480, Honolulu, HI 96826 Phone: 808-949-2208

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

- To create a carrier for neural cells attachment by coating biopolymers with DLC for transplantation.
- 2. To use biodegradable polymer and coating it with DLC for the purpose of carrying neural cells for transplantation.
- 3. To embed the polymer with adhesive molecules/growth factors and then coating it with DLC to enhance neural cells attachment and growth and use it for cell transplantation.

Title: 3A - System for Nerve Cell Function Monitoring

Background

Brief description of what this patent describes:

This patent describes the creation of a self contained semi-solid polymer/gel block which contains nutrients and survival factors to sustain the growth of neurons to couple to a CCD chip for the detection of action potential signals.

What problem(s) does this address?

When the neurons are grown on the CCD chip immersed in a liquid medium environment, attempts to measure the action potential via two electrodes introduced into the fluid causes shorting of the chip. The semi-solid polymer block will allow the measurement without shorting out the chip.

How is this different from what's been done by others?

Currently neurons are cultured in liquid medium. Therefore the CCD chip on which the neurons are grown onto is immersed in liquid.

What future applications might this have?

Self contained units which can house and sustain the growth of neurons can be coupled into bio-driven computer chips.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Chip or CCD itself and its ideal characteristics for detection function.

A water-proof chip is needed for the culture of neural cells on it for detection of signals generated from neurons undergoing stimulation.

Alternatively, a self contained polymer/gel mixture with nutrients and survival factors for neurons can be used for coating the chip surface. The neurons will be cultured in the polymer/gel matrix until they are well developed, then the whole layer will be attached to the chip. assuming that the neurons can survive a period of time (up to several days) within this device, the action can be measured without shorting out the chip (since it does not immerse in fluid). The device is capable of being used as detection tool for nerve gases and other functions.

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Patent Owner: Cellular Bioengineering, Inc.

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Alternative steps or materials to address potential problems or if certain materials are not available:

In order to induce the dendrites of the neurons to make contact with the chip, the surface of the chip can be pre-coated with Nerve Growth (NGF) or DLC.

Better methods or compositions if new materials or complementary methods become available:

A method to supply nutrients and survival factors for the neurons in the polymer/gel unit (by injection, for example) can lengthen the operational period of the polymer/gel unit.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

- 1. To create a semi-solid, self-containing polymer/gel unit for the growth of neurons.
- 2. To couple the polymer/gel unit with neurons grown on it with a CCD chip for detection of action potential after a stimulus is applied.
- 3. To coat the surface of the CCD chip with neural tropic substances (NGF and DLC) to enhance the contact between the dendrites and the chip.

Title: 3B - Composition and Method for Growth of Neural Cells on Various Surfaces

Background

Brief description of what this patent describes:

This patent describes a method to create a polymer/gel matrix with adhesion proteins/growth factors incorporated into it for supporting the growth, replication and differentiation of neural cells.

What problem(s) does this address?:

The growth of neural cells with contemporary tissue culture methods is highly unsuccessful. This problem may be solve by using a three dimensional substrate to embed the neural cells instead of growing them on a flat surface.

How is this different from what's been done by others?;

Most of the current attempts to grow neural cells are in a flat surface.

What future applications might this have?

The ability to grow neurons in tissue culture will make it possible to perform neural cell transplantation. The neural cell cultures can also be used in drug discovery and drug testing, and to test and elucidate the mechanism of infections of neural tissues by micro organisms and viruses.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Use of polymer to grow retinal ganglionic cells for chip technology combined with:

1. "May polymer" (any of the polymers created by the research group of May Griffith PhD, MBA, University of Ottawa Eye Institute, Ontario Canada)

Inventor: Ge Ming Lui Patent Owner: Cellular Bioengineering, Inc.

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May Griffith's polymer was reported to induce dendrite outgrowth from the nerve cells surrounding the cornea. This polymer may provide a three dimensional environment for the neurons to extend their dendrites or even proliferate. May has not shown evidence of nerve cell replication on this polymer yet. To test the possibility, retinal ganglionic cells are readily available and this cell type can replicate at lease 3 to 4 generations on BCE-ECM. The polymer can be used to grow this cell types and see if they can induce proliferation as well as neuronal differentiation.

2. "May polymer" + growth/attachment cocktail.

May polymer by itself may not be sufficient to support the growth, replication and differentiation of neuronal cells. When it is transplanted into the cornea, it may induce the extension of the dendrites (a tropic effect) but the body of the nerve cells are still situated in the surrounding host tissue. To enable the neurons to inhibit the polymer, a growth factor/attachment factor cocktail can be incorporated into the polymer during synthesis. The growth factors suitable for this purpose are bFGF at 50 ug/ml conjugated to polycarbophyll or heparan sulfate and NGF at 50 ug/ml also conjugated to polycarbophyll or heparan sulfate. The attachment factors needed are laminin at 500 ug/ml and RGDS at 500 ug/ml. These components can be added to the collagen type IV (which made up a large part of the May polymer) prior to the polymerization step.

3. "ideal polymer"

If another polymer (one that can be defined better structurally than the May polymer) is available, then it can be tested for the ability for supporting the growth, differentiation and proliferation of cells from neuronal origin. The same approach will be used for its application with the chip technology, i.e. to develop a coat or carrier to house the nerve cells for a period of time long enough for detection of signals.

4. "ideal polymer" + growth/attachment cocktail.

This is the same approach as in adding the growth factors/attachment factors onto the May polymer.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

1. To make a polymer/gel matrix with adhesive proteins/growth factors incorporated into it to provide a three dimensional environment for the growth, proliferation and differentiation of neural cells.

Title: 3C - Composition and Method for Combining Polymers and Diamond Like Coatings for Cell Growth

Background

Brief description of what this patent describes:

This patent describes the coating of biopolymers with Diamond Like Carbon (DLC) for the culture of neural cells.

What problem(s) does this address?

The current tissue culture techniques does not allow for the growth of neural cells in vitro. This problem can be addressed by providing a three dimensional environment for neural cell growth. The use of DLC coating may enhance the attachment and proliferation of the neural cells in culture.

How is this different from what's been done by others?:

Attempts to culture neural cells at present are using regular tissue culture dishes or flasks without the benefit of neurotropic coating.

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Patent Owner: Cellular Bioengineering, Inc.

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What future applications might this have?:

The DLC coated polymer can be molded into the forms of small beads or thin sheets.

These devices can act as carriers for neural cells transplantation.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Use of any of polymers above with DLC.

The polymer can provide a three dimensional matrix for nerve cells to grow, the DLC will help the nerve cells to attach and proliferate. Combining the two technologies together may further enhance the culturing of cells derived from neural origin. The idea is to make a sheet or tiny beads with either the May polymer or another polymer of choice. The polymer sheet/bead will then be coated with DLC (assuming that the polymer can withstand the coating process) and the neural cells will be grown on the coated polymer. The sheet/bead can serve as carriers for cell transplantation in a lot of occasions.

Better methods or compositions if new materials or complementary methods become available:

To incorporate neurotropic factors (such as NGF and bFGF) into the polymer may further enhance the attachment, growth and differentiation of the neural cells.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

- 1. To coat a biopolymer with DLC for use as carrier for neural cell transplantation.
- 2. To coat biopolymers containing neurotropic factors (NGF and bFGF) with DLC for use as culture device or carrier of neural cells in transplantation.

Title: 3D -Composition and Mathod for Combining Polymona and Enter College

Title: 3D -Composition and Method for Combining Polymers and Extra Cellular Matrixes for Cell Growth

Background

Brief description of what this patent describes:

This patent describes the coating of biopolymers with extracellular matrix generated from bovine corneal endothelial cells for the growth of neural and other cell types.

What problem(s) does this address?:

This invention addresses the difficulties of growing human neural cells by conventional tissue culture techniques in vitro.

How is this different from what's been done by others?

Current approach to grow human neural cells involves using tissue culture dishes and flasks without extra coating.

What future applications might this have?

The ability to induce the growth and replication of neural cells in culture may make possible the banking of such cells for transplantation purpose in treatment of neural injury and disorders.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Inventor: Ge Ming Lui
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Use of any of polymers above with ECM.

In a sheet or block form, the polymer can be coated with BCE-ECM for the growth of some neuronal cells. In order to achieve this aim, the polymer will have to be non-swollen when it is submerged in the culture media. The procedure calls for growing of the BCE cells on the polymer sheet/block for 7-10 days, then use 20 mM NH4OH to remove the BCE cells, leaving the ECM coated on the surface of the polymer. The resultant product can be used for neural cells as well as epithelial cells culture.

Better methods or compositions if new materials or complementary methods become available:

Addition of neurotropic factors (such as NGF and bFGF) into the polymer during synthesis may enhance its ability to support the growth and proliferation of neural cells.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

The coating of biopolymers with extracellular matrix derived from bovine corneal endothelial cells
to support the growth and differentiation of neural cells.

 The coating of biopolymers containing neutropic factors (NGF and bFGF) with extracellular matrix derived from bovine corneal endothelial cells to support growth, proliferation and differentiation of neural cells.

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October 6, 2003

Dr. Hank Wuh
Cellular Bioengineering, Inc.
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Re: Draft Claims for Application 5

Compositions and Methods for Cell Culturing

Our Ref. No.: 1003-0005

This patent describes the method of coating a polymer surface with Diamond Like Carbon (DLC) to render it useful as carrier for cells derived from neural origin.

Claims for Application 5

Novel Compositions and Methods for Cell Culturing

- 1. An improved surface for the growth an attachment of cells comprising a biopolymer coated with a high quality, hydrogen free diamond-like carbon surface.
- 2. The improved surface of claim 1 wherein the biopolymer is biodegradable.
- 3. The improved surface of claim 1 wherein the biopolymer is in sheet form.
- 4. The improved surface of claim 1 wherein the biopolymer is in micro particle form.
- 5. A method of growing neurons in culture comprising the seeding and growth of neurons on a biopolymer coated with a high quality, hydrogen free diamond-like carbon surface.
- 6. The method of claim 5 wherein the biopolymer is biodegradable.
- 7. The method of claim 5 wherein the biopolymer is in sheet form.
- 8. The method of claim 5 wherein the biopolymer is in micro particle form.
- The improved surface of claim 1 wherein the biopolymer has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate.
- A method of growing neurons in culture comprising the seeding and growth of neurons on a biopolymer made using the method of claim 9.
- 11. An apparatus for detection of neural cell signals comprising:
 - a unit of biopolymer having embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with

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polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes;

- b) an integrated circuit chip or charge coupled device having a means for said neural processes or dendrites to make an electrical connection;
- c) a detector means for measuring the electrical signals from the neurons; and
 - d) a means for attaching said chip to a detector means.
- 12. The apparatus of claim 11 wherein the biopolymer unit is self-contained.
- 13. The apparatus of claim 11 wherein the biopolymer unit is semi-solid.
- 14. The apparatus of claim 11 wherein the integrated circuit chip or charge coupled device has coated onto it during its synthesis, an attachment reagent comprising one or more of the following: Nerve Growth Factor or Diamond-Like-Carbon, to enhance the electrical contact between the neuronal processes or dendrites and the chip.
- 15. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth.
- 16. The growth medium of claim 15 further comprising "May Polymer".
- 17. The growth medium of claim 16 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.
- 18. The growth medium of claim 17 wherein the concentration of bFGF conjugated with polycarbophyll, or heparin sulfate is about 50 mcg/mL, the concentration of NGF conjugated with polycarbophyll, or heparin sulfate is about 50 mcg/mL, the concentration of laminin is about 500 mcg/mL and the concentration of RGDS is about 500 mcg/mL.
- 19. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth coated with Diamond-Like Carbon.
- 20. The growth medium of claim 19 further comprising "May Polymer".
- 21. The growth medium of claim 20 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve

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Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.

- 22. The growth medium of any of claims 19-21 wherein said biopolymer is shaped into beads, sheets or micro-particles.
- 23. A method of transplanting neurons to a recipient host comprising the seeding of the neurons of interest into the growth medium of any or claims 19-21, allowing the neurons to grow to sufficient density, and implantation of the neurons within the growth medium into said host.
- A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth which is coated with BCE-ECM.
- 25. A method for making the growth medium of claim 24 comprising:
 - a) seeding onto said three dimensional growth medium at low density, a population of bovine corneal endothelial (BCE) cells in a culture media suitable for their growth;
 - b) allowing the BCE cells to grow to confluence; and
 - c) aspirating the media and treating the three dimensional growth medium with ammonium hydroxide for a sufficient period of time to remove the cells.
- 26. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth which is coated with BCE-ECM and with Diamond-Like Carbon.
- 27. The growth medium of claim 26 further comprising "May Polymer".
- 28. The growth medium of claim 27 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.
- 28. The growth medium of any of claims 26-28 wherein said biopolymer is shaped into beads, sheets or micro-particles.

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of Information unless it contains a valid QMB control number. Attorney Docket Number **DECLARATION FOR UTILITY OR** First Named Inventor DESIGN Ge Ming Lui COMPLETE IF KNOWN PATENT APPLICATION (37 CFR 1.63) **Application Number** Filing Date Declaration Declaration October 10, 2003 Submitted Submitted after Initial Art Unit With Initial Filing (surcharge Filina (37 CFR 1.16 (e)) **Examiner Name** required) I hereby declare that: Each inventor's residence, mailing address, and citizenship are as stated below next to their name. I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled: Composition and Methods for Cell Culturing (Title of the Invention) the specification of which is attached hereto OR was filed on (MM/DD/YYYY) as United States Application Number or PCT International Application Number and was amended on (MM/DD/YYYY) (if applicable). I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application: I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed. **Prior Foreign Application** Foreign Filing Date **Priority** Certified Copy Attached? Number(s) Country (MM/DD/YYYY) **Not Claimed** Yes Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

[Page 1 of 2]

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

DECLARATION — Utility or Design Patent Application

										
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NAME OF SOLE OR FIRST IN	VENTOR:		ПАР	etition l	has be	en filed	l for thi	s unsigr	ned inventor	
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